study aimed to assess whether another Cl^- channel blocker, anthracene-9-carboxylic acid (A9C), is also affected by channel phosphorylation. A9C blocks I_{ClCa} at positive potentials but paradoxically stimulates the inward I_{ClCa} tail after repolarization to negative potentials (Piper & Greenwood, *Br J Pharmacol* 138: 31-38, 2003). I_{ClCa} was evoked by pipette solutions containing 500 nM free Ca^{2+} with or without 5 mM ATP to alter the state of phosphorylation. Although A9C (1-500 uM) dose-dependently blocked steady state I_{ClCa} at potentials positive to 0 mV in all cell groups, its maximal effect and sensitivity to voltage were enhanced in cells dialyzed with 0 vs. 5 mM ATP. For example, maximal block by 100 uM A9C was 35 and 73%, and $V_{0.5}$ was 110 and 67 mV, in cells with 5 vs 0 ATP, respectively. A9C enhanced I_{ClCa} tail at −80 mV by causing a negative shift in voltage-dependence in both cell groups, with a larger shift occurring in cells dialyzed with 5 mM ATP. Interestingly, 100 but not 500 uM A9C stimulated steady-state $I_{Cl(Ca)}$ at potentials < 0 mV in cells dialyzed with 0 ATP, a potential range where $I_{Cl(Ca)}$ was unaffected in myocytes dialyzed with 5 mM ATP. As with NFA, the complex actions of A9C on I_{ClCa} are influenced by the state of channel phosphorylation and we propose the existence of at least two binding sites with different affinities for A9C.

645-Pos

F233A Mutation in AT_1R Interrupted Caveolae Targeting and Abolished Regulation of hSlo Channel by Angiotensin II

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The large conductance Ca^{2+}-activated K^+ (BK) channels play an important role in the regulation of vascular tone in response to changes in intracellular metabolic status and Ca^{2+} homeostasis. Angiotensin II (Ang II)-mediated c-Src activation is known to inhibit the activities of BK channels. Recently, trafficking of the Ang II type I receptor (AT_1R) into caveolae was shown to be essential for Ang II signaling and activation of c-Src. We found that BK channels and the AT_1R signaling complex are colocalized in the caveolae of vascular smooth muscle cells (VSMC). In this study, we examined the role of caveolae in the Ang II-mediated modulation of BK channels by co-expressing hSlo channels, AT_1R, caveolin-1, and c-Src in HEK293 cells. Immunoblot analysis confirmed that hSlo and AT_1R were co-precipitated by anti-caveolin-1 antibody only in cells co-transfected with caveolin-1, but not in those without caveolin-1, suggesting that hSlo, AT_1R, and caveolin-1 were physically associated. Exposure to Ang II (2 μM) inhibited the hSlo current density by 32.7 ± 2.8 %, and the Ang II effect was blocked by Losartan (2 μM) with only 1.2 ± 7.8 % current inhibition. However, in cells coexpressing hSlo, c-Src, caveolin-1, and the AT_1R F233A mutant, which abolished its interaction with the caveolin-1 scaffolding domain, exposure to Ang II produced only 6.0 ± 3.7% hSlo current inhibition suggesting that caveolae targeting of AT_1R is crucial for Ang II-mediated BK channel regulation. These results were confirmed by experiments using mouse VSMC. Ang II produced 40% inhibition of BK currents in cells from wt mouse but had no effect on those from cav-1(-/-) animals. Hence, translocation of AT_1R into caveolae upon agonist activation represents a critical step in Ang II regulation of BK channels and vascular function.

646-Pos

Angiotensin II Effects on BK Channel in Mesenteric Arterial Smooth Muscle Cells of SS, BN, and Congenic Renin+ and Renin- Rats

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Cardiovascular sensitivity to anesthetics has been linked to the renin-angiotensin system (RAS), and it is thought to be related to the differences in cardiovascular collapse observed between SS and BN rats. In a previous study we found that congenics carrying the BN renin allele (renin+) in the SS background had the same cardiovascular sensitivity and low BK channel activity as BN rats. On the other hand, congenics carrying the SS renin allele (renin-) displayed high BK activity and behaved like SS rats, suggesting that RSA could be involved in differential responses. To test this hypothesis, the inhibition of BK channel by angiotensin II (AngII, 100 nM) was evaluated in four strains. Activity of BK was monitored from isolated mesenteric arterial smooth muscle cells of SS and BN rats, and renin+ and renin- congenic rats in the cell-attached mode at +80 mV Em and in symmetrical 150 mM K+. Blockade by paxilline (1 μM) confirmed identity of BK channel. Similar to findings from our previous study, in the cell-attached mode the probability of BK channel opening (Po) was different between SS (high Po) and BN (lower Po). The BK activity of renin2- resembled that of SS, whereas BK activity of renin+ matched BN. AngII had a greater inhibitory effect on channel Po in BN (−55+7%) and renin+ (−94+2%) strains than in SS (−9+6%) and renin- (−7+2%) strains. Impaired renin expression and impaired RAS are associated with lower sensitivity of BK to inhibition by AngII in SS than in BN rats. The fact that renin+ and renin- strains follow a similar pattern appears to support this conclusion.

647-Pos

Maxik Channel Beta1 Subunit Interacts and Regulates Thromboxane A2 Receptor Function

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MaxiK channel, composed of the pore-forming α (MaxiKα) and regulatory β1 subunits, controls vascular tone via the activation or inhibition of its pore conducting activity. Thromboxane A2 receptor (TPR), a G-protein coupled receptor, induces potent vasoconstriction mediated by its agonist, thromboxane A2. Previously, we demonstrated that U46619, the stable analogue of thromboxane A2 inhibits MaxiK channel in vascular smooth muscle cell contributing to U46619-induced vasoconstriction. In this study, we report that this inhibition is reversed by nanomolar dehydrosoyasaponin I (DHS-I), a pharmacological tool that indicates MaxiKα and β1 association and functional coupling. The reversing effect of DHS-I indicated that the MaxiK channels inhibited by U46619 could be coupled with regulatory β1 subunits. Co-immunoprecipitation and double immunolabeling in co-expressing cells showed that TPR form a complex with β1 on the plasma membrane. To identify the interacting sites in β1 responsible for TPR-β1 complex formation, we prepared serial carboxyl-terminal deletions of β1 and analyzed their interaction properties with TPR in co-immunoprecipitation experiments. β1 lacking amino acids 103-191 reduces the TPR-β1 association by 44 ± 14% ($p<0.01$), while deletion of residues 73-191 completely reduces the TPR-β1 interaction, suggesting that amino acids 73 to 191 predominantly contribute to the TPR-β1 interaction. To further investigate how β1 regulates TPR-MaxiKα functional coupling, inside-out patch clamp experiments were performed in HEK293T cells expressing TPR + MaxiKα +/- β1 subunit. We found that the β1 subunit reduces U46619-induced MaxiKα inhibition in a dose-dependent manner. In summary, β1 interacts with TPR forming a tripartite complex with MaxiKα and opposes to TPR agonist-induced MaxiK channel inhibition serving as a buffer to vasoconstriction. Thus, in pathological situations like hypertension or aging where β1 expression is compromised the TPR-MaxiK complex would induce severe vasoconstriction. Supported by NIH and AHA.

648-Pos

Kca3.1 Blockers as Potential New Drugs for the Prevention of Renal Fibrosis and Chronic Allograft Rejection

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The calcium-activated potassium channel $K_{Ca}3.1$ is critically involved in the proliferation and migration of T cells, macrophages, dedifferentiated vascular smooth muscle cells and fibroblasts by regulating membrane potential and calcium influx. $K_{Ca}3.1$ has therefore been suggested as a potential therapeutic target for various diseases where activation and excessive proliferation of one or more of these cell types is involved in the pathology. Using the selective small molecule $K_{Ca}3.1$ blocker TRAM-34 as a pharmacological tool compound we previously demonstrated that $K_{Ca}3.1$ blockade prevents restenosis in both rats and pigs and reduces atherosclerosis development in $ApoE^{-/-}$ mice. We now used two models of chronic allograft rejection and one model of kidney fibrosis to evaluate whether $K_{Ca}3.1$ blockers might also be useful for the prevention of transplant rejection and fibrotic kidney changes. In a murine model of obliterative airway disease, where tracheas from CBA mice were heterotopically transplanted into the greater omentum of C57Bl6 mice, both genetic deficiency or pharmacological blockade of $K_{Ca}3.1$ with TRAM-34 reduced luminal obliteration from 92 ± 7% to 60 ± 29% or 61 ± 28% (n = 6 per group). We further performed orthotopic aortic transplantations in the PVG-to-ACI rat model and evaluated chronic allograft vasculopathy after 120 days. TRAM-34 at 10 mg/kg (−35%) and 40 mg/kg (−60%) dose-dependently reduced chronic aortic luminal obliteration. Genetic disruption of $K_{Ca}3.1$ and pharmacological blockade also reduced fibrotic marker expression, chronic tubulointerstitial damage, collagen deposition and alphaSMA(+) cells in kidneys following unilateral ureteral obstruction in mice. Taken together, our findings suggest that $K_{Ca}3.1$ channels are involved in the pathology of obliterative airway disease, chronic allograft vasculopathy and fibrotic kidney disease.
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